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Detection and quantitation of synthetic cannabinoid receptor agonists in infused papers from prisons in a constantly evolving illicit market

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Abstract

Drug misuse in prisons contributes to increased disruption and violence and negatively impacts prisoner safety, rehabilitation, and recovery. Synthetic cannabinoid receptor agonists (SCRAs), colloquially known as 'spice', are infused into papers and are of particular concern in a prison setting where they are commonly vaped. Methods for the qualitative and quantitative analysis of SCRA infused papers, including impurity profiling, were developed using gas chromatography-mass spectrometry (GC-MS) with qualitative confirmation by ultra high pressure liquid chromatography with photodiode array and quadrupole time of flight mass spectrometry detection (UPLC-PDA-QToF-MS) and applied to 354 individual seized paper samples originating from 168 seizures from three Scottish prisons. Of these samples, 41% (146 samples from 101 seizures) contained at least one SCRA and multiple SCRAs were detected on 23% of these papers. Concentrations ranged from <0.05-1.17 mg/cm^2 paper, representing

the first reported quantitative data for SCRA infused papers. An evolution in the SCRAs detected was demonstrated; 5F-MDMB-PINACA (5F-ADB) predominated until late 2018 after which time 5F-MDMB-PICA and 4F-MDMB-BINACA became increasingly more prevalent followed by the arrival of MDMB-4en-PINACA in June 2019. Concentration mapping data from two seized paper samples demonstrated that SCRA concentrations across larger papers were highly variable (0.47-2.38 mg/cm^2 paper) making consistent dosing by users, and representative sampling by laboratory analysts, difficult. Near real-time qualitative and quantitative information on SCRAs circulating in prisons acts as an early warning system for SCRAs emerging on the wider illicit market, inform the methods used to detect them and limit supply, and provide information to support harm reduction measures.

1. Introduction

The reduction of drug misuse and drug harms in prisons has been described as one of the great challenges facing the criminal justice system[1]. Drug misuse contributes to increasing levels of disruption, violence, and crime and has a negative impact on prisoner safety, rehabilitation, and recovery[2-4]. The increased prevalence of potent new psychoactive substance (NPS) use in prisons in the last decade is of particular concern[1,2,5] and is widespread across Europe[6]. The prevalence of synthetic cannabinoid receptor agonists (SCRAs), often referred to colloquially and collectively as 'spice', in prisons in England and Wales is well established and can be described as endemic and entrenched[2,5,7-14]. The actual substances will vary and change over time, presenting analytical challenges for field deployed detection systems and laboratories tasked with detecting and quantifying them for judicial, intelligence, and harm-reduction purposes.

SCRAs are a structurally diverse class of compounds that interact with human cannabinoid type 1 and type 2 G-protein coupled receptors (GPCRs), CB_1 and CB_2[15-19]. They vary widely in their potency and efficacy[20-22] as a result of differences in their structural conformation, including chirality[23]. Their diversity is due, in part, to the increased online availability of published research studies and patents describing their synthesis, in vitro potency and efficacy, and biological effects; the availability of precursor materials; increasing understanding of their structure-activity relationships by producers and suppliers; and as a response to the implementation of national and international legislation designed to control their production, prevalence, and use, and in particular, their use in prisons[16].

SCRAs first appeared in the scientific literature and patents as research tools and potential therapeutic agents, with research in this area continuing today[16]. SCRAs were first formally identified in herbal blends sold for recreational use (commonly referred to as legal highs) in 2008[24]. Until 2016, such normally inert herbal materials infused or sprayed with SCRAs were openly sold by retailers, often referred to as 'head shops' in the United Kingdom (UK) and elsewhere[25], as well as being sold by online vendors.

In 2009 and 2013, two consecutive amendments to the Misuse of Drugs Act (MDA) 1971, the principle legislation in the United Kingdom (UK) for the control of drugs with a potential for misuse and harm, were enacted[26,27] defining analogue controls for SCRAs designed to make the production, possession, and supply of a large number of structurally related compounds illegal. Although helpful in reducing the prevalence of the SCRAs defined in the legislation, this effectively led to a 'cat and mouse' game between producers, sellers, and legislators.

Producers continued to alter SCRA chemical structures to circumvent the legislation and/or evade detection[16]. This, as well as the enactment of other national and international legislative controls, has led to a proliferation of new SCRA compounds, with 260 SCRAs being reported to the United Nations Office for Drugs and Crime (UNODC) by December 2018[28] and over 180 to the EU Early Warning System. The rate of the emergence of new compounds may be slowing[29], but there has been a general trend of increasing potency as the understanding of SCRA structure-activity relationships has improved[17,18,30,31].

In an attempt to end the 'cat and mouse' game, the Psychoactive Substances Act (PSA) was enacted in May 2016 in the UK, making the production, distribution, sale, supply, and possession in custodial institutions (e.g. prisons) of psychoactive substances for human consumption illegal[32], irrespective of whether or not they were covered by the MDA, 1971. In December 2016, a third SCRA-related amendment to the MDA, 1971 ensured the inclusion in the analogue controls of many of the then emerging and potent indazole/indole-3-carboxamide based SCRAs which continue to be prevalent today[20,33]. The analogue controls set out in the 2016 amendment were further amended in November 2019 to reduce the scope of the definition of third generation SCRAs and exclude some compounds that were unintentionally controlled in 2016[34].

The PSA, along with the enforcement of trading standards legislation, effectively led to the cessation of the open sale of NPS, including SCRAs[25]. Whilst clearly reducing the highly visible sale of such substances by retailers, the PSA appears to have had a limited effect on their prevalence of use in some user sub-groups, particularly rough-sleeping and prison communities. In Scotland, since the cessation of their open sale, the use of SCRAs in the general population appears to have decreased rapidly, but their use remains prevalent within the Scottish prison system. Scottish prison survey data from 2017 details that 18% of prisoners report having used NPS prior to entering prison, compared to 27% in 2015, and of these 70% reported the previous use of SCRAs. In 2017, 18% of prisoners reported using NPS whilst in prison, compared to 11% in 2015, and of these, 78% stated they had used SCRAs[35]. While these figures are likely lower than the actual use of NPS and SCRAs in the prisons due to response biases, they may demonstrate a shift in the use of NPS in and outside prisons only a year after the enactment of the PSA, where the use of NPS prior to entering prisons decreased and their use whilst incarcerated increased.

The increase in NPS use has been linked to an increase in violence within Scottish prisons. The Scottish Prison Service (SPS) Annual Report 2017/18 reported an increase in serious 'prisoner on staff' assaults and 'prisoner on prisoner' assaults, and this was linked, in part, to increasing numbers of inmates taking NPS (most likely SCRAs, but not exclusively, as very little data on the compounds circulating was available at that time). There was also a 50% increase in minor or no injury 'prisoner on staff' assaults reported from the previous year, which 'appears to be as a result of an increased unpredictability in prisoners' behaviour'[35]..

SCRAs have been detected in herbal material, powders, e-liquids for vaping, and more recently, infused papers and other materials[11-14, 36-40]. Between December 2014 and June 2015, the most prevalent SCRAs (and/or their metabolites) detected in both urine samples from prisoners and in drug seizures from prisons in England were 5F-AKB48 **(1)**, (*N*-(adamantan-1-yl)-1-(5-

fluoropentyl)-1H-indazole-3-carboxamide), also known as 5F-APINACA, and MDMB-CHMICA **(2)** (methyl 2-[[1-(cyclohexylmethyl)indole-3-carboxamide]-3,3-dimethylbutanoate)[11]. Structures of SCRA compounds discussed in this study are provided in Figure 1 and numbers in bold parentheses refer to these structures throughout the text. The seized SCRA samples were almost exclusively herbal materials sprayed or infused with SCRAs. In their report covering the period 2016-2017, the Forensic Early Warning System (FEWS), coordinated by the UK Home Office and including the analysis of SCRAs in UK prisons, reported the most commonly detected SCRAs to be 5F-MDMB-PINACA **(3)** (methyl 2-(1-(5-fluoropentyl)-1H-indazole-3-carboxamide)-3,3-dimethyl-butanoate) and MDMB-CHMICA **(2)**[12] illustrating changes in SCRA availability in the market over time, most likely as a result of national and international controls. A shift from SCRA impregnated herbal materials (64% of submitted samples) to papers and card sprayed with, or soaked in, SCRA containing solutions (14% of submitted samples), was observed, likely in response to the implementation of prison smoking bans in England and Wales and to facilitate smuggling[12]. This is similar to the ways in which blotters, also known as 'tabs', containing hallucinogens, such as d-lysergic acid diethylamide (LSD) and hallucinogenic NPS, have been prepared for some time[41], although such substances are prepared for sub-lingual use rather than smoking or vaping.

In July 2017, the SPS began implementing a smoke-free policy in Scottish prisons, to be in effect by the end of 2018[42]. Until the end of December 2018, SPS provided free e-cigarette kits to inmates, and until April 2019, inmates could buy e-cigarette kits at a discounted price. Before the smoking ban, inmates either smoked herbal material mixed with tobacco or would roll up a piece of the SCRA-saturated paper into a cigarette and smoke it, but since the ban, inmates are now known to place pieces of SCRA-infused paper between the heating element and the e-liquid cartridge of the e-cigarette. The potential for differential effects of inhaling SCRAs in this way, compared to smoking/pyrolysis, is yet to be explored.

As an acknowledged producer and/or exporter of SCRAs[43,44], it is noteworthy that when the People's Republic of China legislatively controls a specific compound, that compound quickly disappears from the market and is often replaced soon after with new or alternative substances[45,46]. Early in 2019, the State Council of the People's Republic of China introduced analogue controls for a family of potent synthetic opioids (fentanils)[47,48], leading the market to respond with the production of a number of relatively obscure synthetic opioids from different opioid classes. SCRAs, however, continue to be controlled on a compound-by-compound basis. On 29 August 2018, the State Council of the People's Republic of China controlled 32 NPS, additional to those previously controlled, including eight SCRAs[49,50]. These included two of the most prevalent and potent SCRAs on the UK market at that time, 5F-MDMB-PINACA **(3)** and AMB-FUBINACA **(4)**[12,13], as well as ADB-FUBINACA (*N*-(1-amino-3,3-dimethyl-1-oxobutan-2-yl)-1-(4-fluorobenzyl)-1H-indazole-3-carboxamide). Less prevalent indazole-3-carboxamide compounds such as AMB-CHMICA (MMB-CHMICA), ADB-CHMINACA (MAB-CHMINACA; *N*-[1-(aminocarbonyl)-2,2-dimethylpropyl]-1-(cyclohexylmethyl)-1H-indazole-3-carboxamide), and FUB-APINACA (*N*-((3s,5s,7s)-adamantan-1-yl)-1-(4-fluorobenzyl)-1H-indazole-3-carboxamide) were also included as were the indole-3-carboxylate SCRA NM-2201 (naphthalen-1-yl 1-(5-fluoropentyl)-1H-indole-3-carboxylate) and BIM-2201, also known as FUBIMINA ((1-(5-fluoropentyl)-1H-benzo[d]imidazol-2-

yl)(naphthalen-1-yl)methanone). In response, due to the lack of analogue controls, producers have generally responded by introducing structurally similar compounds within established and prevalent structural classes that require minimal changes to existing precursors and synthetic routes, whilst retaining a similar potency and/or efficacy.

This study reports the development of qualitative and quantitative methods for the detection and confirmation of SCRAs in infused papers using gas chromatography-mass spectrometry (GC-MS), and ultra-pressure liquid chromatography with photodiode array and quadrupole time of flight mass spectrometry detection (UPLC-PDA-QToF-MS). The methods were applied to the analysis of paper samples suspected to be infused with SCRAs seized from three Scottish prisons between June 2018 and September 2019. To the best of the authors knowledge, this is the first reported quantitative analysis of seized SCRA infused papers. The study aims to demonstrate the utility of testing such non-judicial samples for monitoring and intelligence purposes, study the effect of legislative changes in SCRA producing jurisdictions on Scottish prison illicit drug markets, improve in-field detection, determine prevalence, and ultimately reduce supply and harms as a result of SCRA use in prisons.

2. Materials and Methods

2.1. Materials

All solvents used were HPLC grade (≥ 99.8% purity) and supplied by either Fisher Chemicals, UK or VWR Chemicals, UK. Tridecane (≥ 99% purity) was supplied by Sigma Aldrich, UK. Ultra-high purity water (18 $M\Omega cm^{-1}$) was obtained using a Milli-Q water purification system (Merck, UK).

2.2. Seized samples

The samples described in this study were non-judicial samples seized by the Scottish Prison Service. Some samples were seized from prisoners directly, as a result of cell searches, or were identified during screening of incoming mail items using portable ion mobility spectroscopy (IMS) systems as previously reported in German prisons and which are becoming increasingly common in UK prisons[37]. Immediately after seizure, samples were placed into tamperproof polythene evidence bags and stored securely. Once it was determined that the samples were not required for judicial proceedings, they were set aside for this study. Prior to sample uplift the items were reviewed by Scottish Prison Service staff to ensure that all personal information present on the seized materials or on the packaging was removed or redacted. Samples were uplifted by staff from the Police Scotland Statement of Opinion (STOP) unit and transported securely to our laboratory. Examples of the items submitted are shown in Figure 2.

2.3. Reference Standards

(*S*)-5F-MDMB-PICA **(5)** (methyl N-{[1-(5-fluoropentyl)-1H-indol-3-yl]carbonyl}-3-methylvalinate) and (*S*)-AMB-CHMICA **(6)**, also known as (*S*)-MMB-CHMICA, (methyl 2-{[1-(cyclohexylmethyl)-1H-indol-3-yl]formamido}-3-methylbutanoate) reference standards were obtained from Chiron, Norway (>99% purity). The reference standard for 4F-MDMB-BINACA **(7)** (methyl 2-[[1-(4-fluorobutyl)indazole-3-carbonyl]amino]-3,3-dimethyl-butanoate) was originally obtained by extraction of the compound from a seized infused paper sample (see Figure 2(a)) using $CDCl_3$, as at the time of analysis, no reference standards were commercially available. Approximately 23 mg of 4F-MDMB-BINACA **(7)** (>98% purity as

assessed by GC-MS and HPLC-DAD) was recovered from this seized paper and identification was confirmed using nuclear magnetic resonance (NMR) and UPLC-QToF-MS (see supplementary information for characterisation data). A (*S*)-4F-MDMB-BINACA **(7)** reference standard purchased from Chiron, Trondheim, Norway (>98% purity) was purchased as a quality assurance check standard once it became commercially available. Reference standards for (*S*)-5F-MDMB-PINACA **(3)** (99.6% purity); (*R*)-5F-MDMB-PINACA (99.6% purity); and (*S*)-AMB-FUBINACA **(4)** (>98% purity) were obtained via in-house synthesis as detailed previously[23]. In addition, (*S*)-5F-MDMB-PICA **(5)**, (*S*)-AMB-CHMICA **(6)**, (*S*)-4F-MDMB-BINACA **(7)**, and (*S*)-MDMB-4en-PINACA **(8)** were synthesised in house as part of this study and characterised using GC-MS and NMR spectroscopy (see supplementary information for synthetic methods and characterisation data).

2.4. Calibration Standards

A series of calibration standards (5-100 μg/mL) were prepared from a 1 mg/mL standard in methanol. Five mL of the 1 mg/mL standard was made by adding 5 mg of the SCRA reference standard(s) to a 5 mL volumetric flask. Five mL of MeOH was added to the flask and the mass was noted, so the actual concentration could be calculated. The solution was transferred to a vial and immediately sealed. All calibration standards were prepared in 5 mL batches in volumetric flasks with 75:25 DCM:MeOH and 0.5 mL of 378 μg/mL tridecane added as an internal standard to give a final internal standard concentration of 37.8 μg/mL. In order to limit DCM evaporation, the standards were first divided into two GC vials that were immediately capped. A 50 μL glass syringe was then used to pierce the GC vial cap and withdraw ten 50 μL aliquots which were then transferred to amber GC vials fitted with 150 μL GC vial inserts. All calibration standards were stored in the freezer until use. Standards (and sample extracts) were injected only once per vial on the GC-MS.

2.5. Instrumental Analysis

NMR spectroscopy for the 4F-MDMB-BINACA **(7)** extracted from the paper sample was performed using a Bruker AVANCE III HD 500 MHz spectrometer (Bruker, Billerica, MA, USA) running under TopSpin v.3.2.5 and equipped with a QCI-F cryo-probe at a sample compartment temperature of 20°C. Samples were prepared in $CDCl_3$ (~10 mg/mL). NMR spectroscopy of in-house synthesised standards reported for the first time in this study (*S*)-5F-MDMB-PICA **(5)**, (*S*)-AMB-CHMICA **(6)**, (*S*)-4F-MDMB-BINACA **(7)**, and (*S*)-MDMB-4en-PINACA **(8)**) was performed using a JEOL ECS-400 NMR spectrometer (JEOL, Tokyo, Japan) operating at 400 MHz for ^{1}H-NMR (10 mg/mL in $CDCl_3$) and ^{13}C-NMR (20 mg/mL in $CDCl_3$).

The GC-MS analysis for both the qualitative and quantitative methods was performed using a 7820A gas chromatograph coupled to a 5977E mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). Injection mode: 1μL sample injection and used either a 5:1 or 20:1 split into a 1mm internal diameter deactivated glass liner pre-packed with quartz wool, injection port temperature: 200ºC, carrier gas: He, flow: 1mL/min. Column: HP-5MS, 0.33μm, 0.2 mm x 25 m (Agilent Technologies). GC oven: 80ºC held for 3min; 40ºC/min to 300ºC held for 3.5 min; total run time: 12 min; transfer line: 295ºC. The mass spectrometer operated in electron ionisation (EI) mode. Ionisation conditions: 70eV in full scan mode (50–550 amu), ion source: 230ºC, quadrupole: 150ºC. For the quantitation of samples with a combination of 4F-MDMB-

BINACA and MDMB-4en-PINACA, a Selected Ion Monitoring (SIM) method was used because these two compounds co-eluted. The same GC method was used as above, but for the MS method, the acquisition type was changed to SIM with two time segments. From 3.00 minutes, the MS scanned for the ions 71.00 (quantitation) and 57.00 (qualifier) for tridecane with dwell time for each ion of 200 ms. From 8.00 minutes, the MS scanned for the ions 219 (quantitation) and 275 (qualifier) for 4F-MDMB-BINACA and 213 (quantitation) and 301 (qualifier) for MDMB-4en-PINACA with dwell time for each ion of 150 ms.

UPLC-PDA-QToF-MS analysis for the qualitative confirmatory analysis of SCRA containing paper extracts was performed using an Acquity UPLC® instrument with a binary pump, autosampler held at 4ºC, vacuum degasser, and column oven held at 30ºC coupled to a Xevo QToF-MS (Waters Corporation, Milford, MA, USA). Mobile phases used were (A) LC-MS grade water with 0.1% formic acid and (B) acetonitrile with 0.1% formic acid. The gradient used was 50:50 A:B from 0.0-4.0 min, 25:75 A:B from 4.0-5.0 min, 5:95 A:B from 5.0-5.99 min, and 50:50 A:B from 6.0-7.0 min. Flow rate was 0.5 mL/min and 2 uL of sample was injected onto a BEH C_{18} 50 × 2.1 mm, 1.7 mm particle size column (Waters Corporation, Milford, MA, USA). The QToF was operated in positive ionisation mode with a source temperature of 120ºC, a desolvation temperature at 500ºC, and a capillary voltage at 2.25kV. ToF-MS analysis for the high-resolution determination of molecular mass was carried out with a collision energy at 6V. MS^e acquisition was carried out using collision energies ranging from 0 to 40 V. After the QToF-MS and MS^e data were processed, accurate parent ion fragmentation spectra were obtained using MS/MS data acquisition of selected parent ion accurate mass data using collision energies between 10 and 30V.

2.6. Preliminary Method Development

Preliminary method development work determined the best solvent for GC-MS qualitative and quantitative analysis. Using 10 repeated standard injections from the same vial, dichloromethane provided the highest peak area response of all the solvents tested, but also had the highest peak area variance due to its volatility (see supplementary information). This was due to the evaporation of the dichloromethane from the pierced vial septum resulting in the SCRA becoming more concentrated and peak areas increasing over the injection series. When the experiment was repeated with multiple single injections from different vials the variance decreased dramatically (see supplementary information). Methanol was chosen as the extraction solvent for qualitative analysis, so that samples could subsequently be analysed using UPLC-PDA-QToF-MS; and 75:25 dichloromethane:methanol (DCM:MeOH) was chosen for quantitative analysis and samples in vials would only be injected once. This solvent choice for quantitation ensured that compounds with a range of polarities could be extracted, provided good GC-MS precision, and allowed the use of methanol as a 'keeper' solvent when preparing calibration standards and quality assurance samples. While a deuterated standard as an internal standard for the quantitative method would have been ideal, at the concentrations used for the quantitation in this study, this would have been prohibitively expensive, and this method was designed to be widely applicable and low cost. Instead, tridecane was used as an internal standard. All screw cap vials were sealed with parafilm or high quality crimped vials were used to minimise any DCM evaporation.

To verify that three sequential extractions was sufficient to extract SCRAs from the paper samples. Three 1x1 cm pieces of blank white paper were impregnated with 75 μL of a 1 mg/mL solution of the SCRA by suspending the paper between a set of micro forceps between a clamp and dripping the solution onto the paper, making sure all of the solution remained on the paper. Once dry, each piece was placed in a glass vial and sequentially extracted 5 times using 75:25 DCM:MeOH and 5 minute ultrasonication. For each piece, each of the five extractions was placed in a separate GC-MS vial and analysed. The peak areas of each extraction were collected, and the percentage of the total peak area determined. Based on the three samples extracted for each SCRA, all of the SCRA was extracted after three extractions. The data is provided in the supplementary information.

2.7. Qualitative Analysis

Where the size of the seized paper/card sample permitted, two approximately 1 cm^2 samples were cut from opposite corners and placed in a glass vial, then 0.25 mL methanol was added, and the vial was capped and sonicated for five-minutes. The extracts were recovered and analysed using GC-MS. This often provided 'overloaded' chromatograms where SCRAs were present, allowing the identification of minor SCRA and non-SCRA related components extracted from the paper to be determined as an exploration of the potential for SCRA batch profiling, except where SCRAs were present only in low concentrations in the extract (equivalent to approx. 5-10 $\mu g/cm^2$ paper depending on the individual SCRA). As no reference standards were available for these minor components and they were often not included in the available spectral libraries, they have only been tentatively identified. Sample extracts were diluted and the peak areas of the minor components were calculated relative to the major component. SCRAs were identified by comparing their retention time and mass spectra to reference standards of known origin and by comparison to NIST14, SWGDRUG (v3.5), and Cayman Chemicals (versions v04262019 and v09112019) mass spectral libraries with a minimum acceptable reverse match value of 850. In the minority of cases where reference standards were not available and/or compounds were not present in the spectral libraries, tentative identifications were made by elucidation of their molecular structure using fragmentation patterns and visual comparison with available online electron impact (EI) ionisation and QToF-MS spectra where available (e.g. Response 2 Project[51], Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) monographs[52], The Center for Forensic Science Research and Education (CFSRE) NPS discovery monographs[53]) and/or relevant peer-reviewed literature. All analyte identifications by GC-MS were orthogonally confirmed by analysis of either a 10 times dilution of the qualitative analysis extract or the undiluted extracts using UHPLC-PDA-QToF-MS in low fragmentation high resolution accurate mass (TOF-MS) and tandem (MS/MS) modes.

2.7. Quantitative Analysis

A 3 mm diameter hole punch sample was collected from previously analysed samples using a 3mm biopsy punch, adjacent to where the qualitative sample(s) had been taken. The collected paper was placed in a screw-cap glass vial, and sequentially extracted three times in 0.25 mL 75:25 dichloromethane:methanol (DCM:MeOH), after which the three extracts were combined. The combined extracts were weighed and the total volume calculated. A 100 μL aliquot of this extract was diluted to 200 μL using 80 μL of 75:25 DCM:MeOH and 20 μL of internal standard (tridecane) solution in 75:25 DCM:MeOH to give a final internal standard

concentration of 37.8 μg/mL. The GC-MS vial was then sealed with parafilm to prevent any solvent evaporation while sitting on the GC-MS sample carousel. The remaining original sample was frozen at -20°C. For 5F-MDMB-PICA, AMB-CHMICA, and other indole-based SCRAs, a 100 μg/mL standard was run on the GC-MS to check for any degradation products as these were seen to increase as the GC liner was used and disappeared when the liner was replaced. The GC-MS was calibrated using three sets of a series of SCRA reference standards (5-100 μg/mL) with tridecane as an internal standard. An average of the three sets of calibration standards were used to generate the calibration curve. The accuracy of the calibration curve was determined using independent calibration check standards at approximately 30 and 85 μg/mL and was approximately 3% with a maximum allowable bias of +/- 5%.

To determine the accuracy of the quantitation method for SCRA infused paper it was necessary to load a known amount of SCRA onto a known area of paper accurately and without loss of SCRA. This was not possible in a way that would directly mimic a 3mm diameter paper sample extraction as used for seized samples in this study, due to the diffusion of the spiking solution through the paper over an area greater than 3mm diameter. Therefore, for each SCRA (except MDMB-4en-PINACA as this analyte was added only in the later stages of this study), seven spiked paper pieces were prepared as previously described for the sequential extraction experiment. A 75 μL aliquot of a 1 mg/mL SCRA solution was added by pipette to a 1 cm^2 piece of paper held between forceps with the solution not diffusing out of this area. Three 250 μL sequential extractions using DCM:MeOH (75:25) were made and the extracts combined. As a result of DCM evaporation and solvent adsorption into the paper the final combined extract was weighed and the total volume calculated (approximately 500-600 μL) giving a solution of approximately 150 μg/mL SCRA, if 100% extraction efficiency is assumed. Two-fold dilutions of each extraction solution were prepared in triplicate. The three sets of the extraction solutions of the seven samples were analysed by GC-MS following the appropriate calibration curve and check standards. The diluted spiked sample extracts gave peak area responses in the middle of the calibration range. The SCRA concentrations on the paper were calculated using a sample area of $1cm^2$ rather than the 3mm diameter circle used for the seized samples (see supplementary information). As a result, although the concentration (mg/cm^2) was lower in the spiked samples, the absolute mass of SCRA extracted was within the range observed for 3mm hole punch samples for seized samples. The mean and standard error of the mean (SEM) for the three replicates of each sample were calculated as well as the mean and SEM of the concentrations across all samples. The expected paper concentration for the spiked paper samples was 75 $\mu g/cm^2$. The mean SCRA concentrations in the spiked samples ranged from 66.13 (-12.6% bias) to 79.20 (+5.1% bias) $\mu g/cm^2$ paper and the SEM ranged from 0.22 to 4.39 $\mu g/cm^2$ with SCRA LODs ranging from 4.1 to 7.9 $\mu g/cm^2$. See supplementary information for the spiking experiment data.

The accuracy of the quantitative method for each batch of samples was checked using a spiked and blank paper sample extracted alongside each batch (maximum of 20 samples) in addition to analysis of the previously described calibration check standards. An example calibration curve and associated data for calibration check standards and the positive batch control samples (spiked paper) is provided in the supplementary information. The percent error of the spiked samples during the sample runs ranged from 1.9-15.2% with an average of 8.6% and median of 11.1%. The estimated percent error of the quantitative method determined from the method

validation was 15% and is provided as a ± after the calculated value. The blank paper sample was a 1x1 cm piece of blank white paper that was placed in a glass vial and extracted alongside all of the other samples. The calculation of the calibration curve and concentrations of samples was performed using an R script. Sample aliquots in inserts within 2 mL amber vials were injected only once. Samples with SCRA peak area ratios outside the upper range of the calibration curve were reanalysed using a greater dilution of the original sample extract. Samples with SCRA peak area ratios below the lower range of the calibration curve were denoted as below the limit of quantitation (LOQ), which was calculated for each sample based on the lowest calibration standard concentration and the volume of the sample's three-extraction solution. LOQs ranged from 0.05-0.09 mg/cm^2 paper.

2.8. Mapping SCRA concentrations across seized papers

Due to the known methods for the illicit preparation of SCRA infused papers and card, SCRA concentrations are likely to vary across infused sheets of paper, making consistent dosing by users almost impossible. The extent of this variation in seized infused paper samples has not previously been investigated. One piece of card, found during qualitative analysis to contain AMB-CHMICA **(6)**, seized from Prison 1 on 5 March 2019 and one set of multiple papers which had originally formed a larger single sheet of paper, found to contain 5F-MDMB-PICA **(5)**, seized from Prison 1 on 7 March 2019 were selected for more detailed quantitative analysis using a method adapted from that described by Angerer, et al. (2018)[54]. A clean A4 sized piece of tracing paper was printed with a 1 cm^2 grid. This grid was cut to size, overlaid, and secured onto the paper to be sampled and was used as a guide to collect a 3 mm diameter punch sample from each grid square. Each 3 mm diameter punch was analysed using the quantitative method described above.

2.9. Laboratory prepared SCRA impregnated paper samples

To study the variability of SCRA concentrations across papers in a more controlled manner, six 5x5 cm (25 cm^2) pieces of lined 80 g/m^2 paper were prepared and pre-gridded into 1 cm^2 sections using a pencil. 20.1 mg of a previously synthesised[23] (*R*)-5F-MDMB-PINACA **(3)** standard was dissolved in approximately 5 mL of ethanol to give a 4.01 mg/mL solution. The solution was poured into a glass beaker and each 5 cm^2 piece of paper was laid flat and soaked in the (*R*)-5F-MDMB-PINACA **(3)** solution for approximately 10 seconds then removed carefully from the solution taking care to keep the paper flat as it was removed from the solution. Three papers (A1-A3) were laid flat to dry on a large glass tile and the other three pieces were hung up to dry, the top of the paper having been marked in pencil prior to soaking in the SCRA solution. The papers were left to dry for 1 hour before each piece was cut into the previously gridded 1 cm^2 sections (25 samples per paper). Each individual square was extracted using the quantitative procedure described above, adapted to account for the difference in paper sample size taken.

3. Results and Discussion

3.1. SCRA market evolution – qualitative and quantitative analysis

From 1 June 2018 to 27 September 2019, 360 individual seized paper samples originating from 168 seizures from three Scottish prisons were analysed. Of these samples, 41% (146 samples from 101 seizures) contained at least one SCRA. Full analytical data (GC-MS and UPLC-PDA-

QToF-MS) for these samples is provided in the supplementary information. The findings are summarised in Table 1 and the variation in concentrations of the five quantified SCRAs and the total SCRA concentration when multiple SCRAs were present in the same sample are shown in Figure 3. Of the 145 individual papers found to contain at least one SCRA, 40% (59 samples) contained 5F-MDMB-PICA **(5)** as a main component ranging in concentration from $<0.08 \pm 0.01$ to 0.76 ± 0.11 mg/cm^2 paper; 31% (45 samples) contained 4F-MDMB-BINACA **(7)** ranging in concentration from $<0.09 \pm 0.01$ to 0.94 ± 0.14 mg/cm^2 paper; 29% (42 samples) contained 5F-MDMB-PINACA (5F-ADB) **(3)** ranging in concentration from $<0.05 \pm 0.01$ to 1.17 ± 0.17 mg/cm^2 paper; 15% (22 samples) contained MDMB-4en-PINACA **(8)**, ranging in concentration from $<0.07 \pm 0.01$ to 0.58 ± 0.09 mg/cm^2 paper; 3% (5 samples) contained AMB-FUBINACA **(4)**, ranging in concentration from 0.20 ± 0.03 to 1.16 ± 0.17 mg/cm^2 paper; and 1% (1 sample) contained AMB-CHMICA **(6)** with a concentration of 0.58 ± 0.09 mg/cm^2 paper. As far as the authors are aware this data represents the first time that SCRA concentrations in seized infused papers have been reported.

Of these 146 samples, 23% (33 samples) contained multiple SCRAs with one sample seized in Prison 1 on the 28th November 2018 found to contain four SCRAs: 5F-MDMB-PINACA **(3)** (major), CUMYL-4CN-BINACA **(10)** (4.4% of 5F-MDMB-PINACA peak area), AMB-FUBINACA **(4)** (4.1%), and 5F-MDMB-PICA **(5)** (1.7%). As no reference standard for CUMYL-4CN-BINACA **(9)** was available in our laboratory, this compound was identified by comparison of spectra (see supplementary electronic information) to published GC-MS and UPLC-QToF-MS data[51-53, 55]. In 11 cases, these other SCRAs were present in very minor proportions (<1% of major SCRA peak area) possibly indicating cross contamination prior to our analysis, whilst in 22 cases they were present in higher proportions, indicating more purposeful addition (Table 2). For example, in April and May 2019 there were two samples detected with an almost 50:50 proportion of 5F-MDMB-PINACA and 5F-MDMB-PICA and 73% (16 samples) of all MDMB-4en-PINACA detections also contained 4F-MDMB-BINACA as a major component. Where multiple SCRAs were detected in the same paper sample, their combined SCRA concentration remained within the concentration range calculated for single SCRAs. A plot of the *total* SCRA concentration in each sample as a function of seizure date is provided in the supplementary information. The timeline of the detection of different SCRAs in Scottish prisons is provided in Figure 4. 5F-MDMB-PINACA **(1)** dominated between June and November 2018, but after this date, different compounds began to be detected including 5F-MDMB-PICA **(5)** in November 2018, which went on to become the most commonly detected SCRA in this dataset; 4F-MDMB-BINACA **(7)** in February 2019; a single sample containing AMB-CHMICA **(6)** in March 2019; and MDMB-4en-PINACA **(8)** in June 2019.

5F-MDMB-PICA **(5)**, 4F-MDMB-BINACA **(7)**, and MDMB-4en-PINACA **(8)** detections increased over the time of the study and the number of samples in which multiple SCRAs were detected also increased. From the data presented, it seems clear that the introduction of legislative controls on the production and export of 5F-MDMB-PINACA **(3)** and AMB-FUBINACA **(4)** by the People's Republic of China on 29 August 2018[49,50], has led to their decreased prevalence in Scottish prisons and the emergence of structurally related indole/indazole-3-carboxamide SCRA compounds, with similar synthetic routes to 5F-MDMB-PINACA **(3)**, not covered by the ban (e.g. 5F-MDMB-PICA **(5),** 4F-MDMB-BINACA **(7)**, and MDMB-4en-PINACA **(8)**).

In Europe, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) notified member states of the first seizures/identifications of 4F-MDMB-BINACA **(7)** in France and the Netherlands in October 2018 and in the UK in November 2018[56]. It was detected in three seized herbal materials (seizure dates unknown) and one small piece of paper (seized following a positive 4F-MDMB-BINACA metabolite detection in a prison sample in January 2019) in Germany[38].

In the UK, 4F-MDMB-BINACA **(7)** was detected by the Welsh Emerging Drugs and Identification of Novel Substances (WEDINOS) service in December 2018 in samples of herbal materials and has also been detected in e-liquids for vaping, purporting to contain THC. Between 14 December 2018 and 22 November 2019, 94 detections of 4F-MDMB-BINACA have been reported by the service. Interestingly, WEDINOS have not, up to 2nd December 2019, reported any detections of 5F-MDMB-PICA **(5)** in publicly available data[39] despite it being the most commonly detected SCRA in this study, indicating possible localised market differences. The first WEDINOS detection of MDMB-4en-PINACA was from a sample submitted on the 14th August 2019 and it has been detected in three further samples, all of which were detected with 4F-MDMB-BINACA[39], similar to the samples described in this study, possibly indicating a potential intelligence link between the materials (or market availability).

Similar trends have been reported in the United States demonstrating a globalised market in SCRA production and export. Krotulski et al.[7] described the first detection of 4F-MDMB-BINACA **(7)** in the United States in seized herbal material in December 2018 and note the substance was first also detected in November 2018 in Singapore[57]. Between November 2018 and March 2019, 4F-MDMB-BINACA **(7)** was detected in 29 toxicology cases. The CFSRE NPS Discovery programme reported that between January 2019 and June 2019, 5F-MDMB-PICA **(5)** and 4F-MDMB-BINACA **(7)** were the most commonly detected SCRAs in casework[58,59]. Prior to that, as in our data, 5F-MDMB-PINACA **(3)** had been the most commonly detected compound with 5F-MDMB-PICA emerging in the third quarter of 2018. MDMB-4en-PINACA was first reported in Europe in a test purchase as part of the RESPONSE 2 project[60] and notified to EU member states via the EU Early Warning System in August 2018[61]. CFSRE reported their first detections of MDMB-4en-PINACA in forensic toxicology casework samples collected in the United States in July 2019[62]. In the United States Drug Enforcement Agency's Special Testing and Research Laboratory's Emerging Trends Program report for quarter 1 of 2019, 5F-MDMB-PINACA **(3)** was the most commonly detected SCRA, followed by 5F-MDMB-PICA **(5)**, which had begun to increase in prevalence from the third quarter of 2018. This programme has not, as of 1 July 2019, reported any 4F-MDMB-BINACA **(7)** or MDMB-4en-PINACA **(8)** detections[63-66].

The evolution of the SCRA market in Scottish prisons can be described as being relatively conservative, with little variability of compounds at any one time and emerging compounds having remained for some time almost exclusively within the indole/indazole-3-carboxamide structural class. It is difficult to predict which new compounds might appear next should there be a market driver for change; however, similar structural analogues of current SCRAs such as AMB-4en-PICA (MMB-2201; Methyl 3-methyl-2-[(1-pent-4-enylindole-3-

carbonyl)amino]butanoate) and MDMB-4en-PICA (methyl-3,3-dimethyl-2-(1-(pent-4-en-1-yl)-1H-indole-3-carboxamido)butanoate) may be likely. SCRAs most recently detected in the European early warning system (EWS), but not in Scottish prisons to date, include the alkylcarboxyl-indazole-3-carboxamide APP-BINACA (*N*-(2-amino-1-benzyl-2-oxo-ethyl)-1-butyl-indazole-3-carboxamide)[67], which has also been detected in toxicology case samples in the United States, commonly alongside 4F-MDMB-BINACA[68]; CUMYL-CBMICA (1-(cyclobutylmethyl)-*N*-(2-phenylpropan-2-yl)-1*H*-indol-3-carboxamide)[69] which is unusual in that it replaces the more commonly seen alkyl/fluorobenzyl 'tail' moiety with a cyclobutylmethyl 'tail' moiety; the adamantyl azaindole 5F-A-P7AICA (*N*-(adamantan-1-yl)-1-(5-fluoropentyl)-1*H*-pyrrolo[2,3-b]pyridine-3-carboxamide)[70]; 2F-QMPSB (quinolin-8-yl 3-((4,4-difluoropiperidin-1-yl)sulfonyl)-4-methylbenzoate), a arylsulfonamide-based synthetic cannabinoid[71]; and the naphthoylindole 5F-JWH-398 (1-(5-fluoropentyl)-3-(4-chloro-1-naphthoyl)indole)[72].

In a similar manner to Δ^9-tetrahydrocannabinol (Δ^9-THC), the major psychoactive component of cannabis, SCRAs exert their cannabimimetic effects primarily through interaction with cannabinoid receptors. All SCRAs detected in this study to date are known or expected to be potent CB_1 agonists, however a range of different in vitro assays to determine CB_1 and CB_2 potency and efficacy have been used in the literature and direct comparisons should be made with caution[73-75]. Using a FLIPR assay which measures changes in membrane potential, Banister et al., (2016) reported similar CB_1 EC_{50} values for 5F-MDMB-PICA **(5)** and 5F-MDMB-PINACA **(3)** (0.45 and 0.59 nM both being more potent at CB_1 than AMB-FUBINACA **(4)** and AMB-CHMICA **(6)** (2.0 nM and 3.5 nM respectively), and all were considerably more potent than Δ^9-THC (171 nM)[74]. As might be expected from available pharmacological data for structurally similar compounds, 4F-MDMB-BINACA has been shown by Jawalosky[76] (as referenced in a recent World Health Organisation Critical Review[77]) to bind to the CB_1 receptor (4F-MDMB-BINACA at CB_1: Ki = 14.3 nM; (R)-(+)-WIN-55,212-2: Ki = 172 nM; Δ^9-THC: Ki =22.5 nM using HEK cells and [3H]CP-55,940 (~1.3 nM) as a radioligand) and to have functional activity as assessed using an adenylate cyclase assay using a cyclic AMP ELISA kit (4F-MDMB-BINACA, EC_{50} = 0.20 nM (E_{max} = 67.7%); (-)CP-55,940, EC_{50} = 0.40 nM (E_{max} = 95.6%); Δ^9-THC, EC_{50} = 14.2 nM (E_{max} = 82.9%)). To the best of the author's knowledge there is currently no publicly available data on the pharmacology of MDMB-4en-PINACA; however, based on existing structural-activity relationships it is highly likely to be a potent CB_1 and CB_2 receptor agonist[20,30,31].

3.2. Impurity profiling

Several impurities were identified in some samples during the initial qualitative screening analysis. Three potential impurities were consistently found in 5F-MDMB-PICA **(5)** containing samples (spectra for these minor components are provided in the supplementary electronic information): a tentatively identified fluorinated PICA (0.4-18% of 5F-MDMB-PICA peak area, detected in 66% of the samples) and tentatively identified 5-fluoropentylindole impurity (0.16-0.35% of 5F-MDMB-PICA peak area), which may be either impurities or degradation products, and a tentatively identified 5Cl-MDMB-PICA (0.3-3.1% of 5F-MDMB-PICA peak area), which is likely a synthesis by-product. Five 4F-MDMB-BINACA **(7)** samples also contained a tentatively identified 4Cl-MDMB-BINACA impurity as a minor component (0.1-1.26% of 4F-MDMB-BINACA peak area), likely to be a synthesis

by-product. Five 5F-MDMB-PINACA samples contained trace amounts of a tentatively identified 5Cl-MDMB-PINACA impurity, likely to be a synthesis by-product. Although often only very minor components, the tentatively identified impurities in the SCRA samples might, alongside chiral analysis, facilitate batch profiling.

Two previous studies have noted degradation of SCRAs which may or not be analytical artifacts[78,79]: degradation of PB-22 **(11)** (quinolin-8-yl 1-pentyl-(1*H*-indole)-3-carboxylate), also known as QUPIC; FUB-PB-22 **(12)** (quinolin-8-yl 1-[(4-fluorophenyl)methyl]indole-3-carboxylate); 5F-PB-22 **(13)** (quinolin-8-yl 1-(5-fluoropentyl)indole-3-carboxylate); 5F-APICA **(14)** (*N*-(1-adamantyl)-1-(5-fluoropentyl)indole-3-carboxamide), also known as STS-135; and 5F-APINACA **(3)** when in methanol or ethanol. It was discussed that the degradation could be thermal degradation during GC-MS analysis or just from the process of dissolution[78,79] and this factor warrants further investigation, specifically for the potent and prevalent indole-3-carboxamide and indazole-3-carboxamides detailed in this study. Breakdown of these compounds in the GC liner over time was noted in this study, which was mitigated by changing the GC liner.

In all four samples where AMB-FUBINACA **(4)** was present as the main SCRA, EMB-FUBINACA **(10)** was detected as a minor component (0.21-0.27% of AMB-FUBINACA peak area). In three of these samples, the synthetic cathinone 4F-PHP (1-(4-fluorophenyl)-2-(pyrrolidin-1-yl)hexanone) was tentatively identified by comparison to EI spectra in the Cayman spectral library and published MS/MS data[80] (see Supplementary Information for spectra), twice as a minor component (<1.0%) in samples seized from Prison 1 (Figure 2(c)) and once as a major component (12.9% of the AMB-FUBINACA peak area) in a sample seized in Prison 3. To the best of the authors knowledge, there have been very few reports of synthetic cannabinoid/synthetic cathinone mixtures and none in seized infused papers. Recently, there was a report of the synthetic cathinone *N*-ethylpentylone found in combination with synthetic cannabinoids in post-mortem urine in four prisoners from Florida between March 2017 and November 2018[80]. While SCRAs have been reported to enter prisons in Florida via impregnated paper, it is unclear from the results of post-mortem urine testing if the mixture was on the paper or if the SCRAs and synthetic cathinones were taken separately. In New Zealand in 2017, of 157 AMB-FUBINACA containing herbal samples, 55 (35%) were found to contain para-fluorophenylpiperazine (pFPP)[81]. It is not currently known why these additional compounds are being added to AMB-FUBINACA infused substrates. These tentatively identified mixtures, impurities and by-products, along with chiral profiling, may prove useful in future batch profiling studies.

3.3. Concentration mapping across seized SCRA infused papers

A typical dose of SCRA infused paper appears to be approximately 1 cm^2 or smaller (Figure 2(d)). This size of paper will fit between the e-liquid cartridge and the heating element in an e-cigarette. There is also evidence that, where available, users will utilise paper punches to create circular dosage units, with this format fitting better into the e-cigarettes than the square samples (Figure 2(d-f)). Such samples are easy to conceal, transport, and exchange between prisoners.

Two larger seized paper samples, from which doses would be created, were selected for a detailed study of SCRA concentration variation across single sheets of paper to indicate the variability of doses from that sheet of paper. The first sample comprised a dark coloured greetings card with two pieces of white card on the inside (Figure 5a). Both pieces of white card, measuring approximately 150x105 mm, bore visible brown coloured wash marks. One

piece of white card was selected at random and a total of 163 individual hole-punched samples were collected using an overlaid 1cm^2 grid and the AMB-CHMICA **(6)** concentrations determined as previously described. The data is summarised using a concentration heat map (Figure 6b) and shows that there was significant variation of concentrations across the card, ranging from 0.47-2.38 mg/cm^2. The highest concentrations were in the middle of the card and the lowest concentrations tended to be in the corners. In this case, the SCRA containing solution used to prepare the card was most likely added to the centre and the AMB-CHMICA **(6)** containing solution moved outwards as the solvent travelled through the paper and evaporated.

In contrast, there was no visible staining on the pieces of paper from the second sample, known to contain 5F-MDMB-PICA **(5).** The sample comprised 12 separate pieces of white paper of varying sizes with black inked handwriting on one side (Figure 6a). Through visual comparison, all 12 pieces were found to have originated from the same letter; however, only six of the pieces formed a physical fit, with the handwriting on these six pieces continuing from the adjacent piece of paper. These six pieces of paper were selected for concentration mapping. In total, 208 individual quantitative analyses were carried out, taking one hole-punch sample of paper per cm^2 and the samples quantitatively analysed. The resultant heat map (Figure 6b) shows a variable distribution of 5F-MDMB-PICA **(5)** across the letter, with the lowest concentration in square 'N2' at 0.48 mg/cm^2 and the highest concentration in square 'B1' at 1.34 mg/cm^2. The highest concentrations were detected in one corner of the paper (if all the paper pieces are considered as a single sheet) consistent with the paper having been soaked and then held at one corner to drip dry and then dried flat or held at one corner and dried hanging up).

To demonstrate the influence of the SCRA infusion and drying method on SCRA distribution across paper, a controlled SCRA paper dosing experiment was carried out using a 5F-MDMB-PINACA **(6)** solution in ethanol. The distribution of 5F-MDMB-PINACA **(6)** in the dried papers (Figure 7) was less variable when the infused papers were laid flat to dry, compared to when they were hung up to dry. In the samples that were hung up to dry, concentrations at the bottom of the papers were considerably higher than the top sections. This clearly demonstrates the influence of preparation method on SCRA concentration variability across sheets of paper. Taking a pragmatic harm reduction-focussed view, preparing SCRA infused papers in a manner that minimises concentration gradients across the paper would at least allow for more consistent dosing across a single sheet.

SCRA heterogeneity has been reported previously in SCRA infused herbal samples[80,81] leading to inconsistent dosing, increasing the likelihood of users experiencing unpredictable effects. In such samples, this variability can be mitigated somewhat by mixing or shaking the herbal material prior to smoking, however this is not possible with an infused paper. The data presented in this study clearly shows that SCRA concentrations can vary considerably across a single sheet of paper, which will then be cut into a series of smaller dosage units and users may be unaware of this variability. This increases the inherent risks of using papers infused with potent psychoactive substances such as SCRAs compared to other available forms of the drug.

Unlike other sample substrates such as powders and herbal materials, SCRA infused papers cannot be homogenised prior to sampling and therefore must be representatively sampled. Obtaining representative qualitative data for SCRAs infused into paper is challenging. This is especially true for larger paper samples as the concentration gradients across the paper surface will be dependent upon; the preparation method and its consistency within and between preparation batches; the size of the sample seized; the proportion and position or the seized sample relevant to the original 'whole' paper sample at the time of preparation. The samples seized in this study ranged from 1 cm^2 'dosage' units to A4 pieces of paper (21.0 x 29.7cm). In this study, we utilised a 3mm hole punch for the quantitative method with the original idea that such a sampling method could potentially be used to sample sealed items of mail without having to open them. However, to obtain more representative quantitative data it is recommended that larger sub-samples are taken in future studies (e.g. 1cm^2) and that multiple samples are taken from across the paper surface (e.g. 4 corners and centre) dependent on and proportionate to the size of the seized paper in question. Sample extracts from these papers could then be combined and diluted prior to analysis. Additionally, there is an opportunity to explore the potential for the application of appropriate mass spectral imaging techniques to study SCRA distribution across papers to ensure representative sampling and this is recommended for future study.

In addition to increased NPS prevalence, the increase in assaults reported in Scottish prisons, could be linked to a change in the compounds present on the SCRA market, with compounds becoming relatively more potent. Additionally, increased variability and unpredictability in dosing due to the use of impregnated papers in prisons or changes in the mode of use of SCRAs, e.g. changing from smoking of herbal material (pyrolysis) to vaping of infused papers using e-cigarettes, may influence the psychoactive effects and harms experienced by users, which warrants further research. Continued vigilance is required to maintain our understanding of the SCRA market in prisons, ensure that field detection systems remain able to detect new SCRA compounds as they appear on the market, and continue to improve harm reduction services. In the short- to medium-term, the implementation of mail scanning using ion mobility spectrometer (IMS) systems and copying procedures for SCRAs may be effective in reducing the supply of infused papers into prisons via the mail system; however, the supply chain may respond in a variety of ways which could also lead to increased harms in the short-term e.g. changing SCRA compounds and shifting the production of SCRAs infused papers into prisons using any solvents available.

4. Conclusions

Methods for the qualitative and quantitative analysis of SCRA infused papers using GC-MS and UPLC-PDA-QToF-MS were developed, validated, and successfully applied to 354 non-judicial paper samples seized from three Scottish prisons between June 2018 and September 2019. Our analysis has confirmed that SCRA infused papers, designed to evade detection and facilitate smuggling, are currently circulating and are highly prevalent within Scottish prisons and both the nature of the substances present and their concentrations are variable both between paper samples and across individual sheets. SCRA concentrations across the whole of two papers studied in detail varied by up to a factor of seven across an individual sheet with the variation due to the methods in which the papers were prepared and dried. A clear change in SCRA prevalence from 5F-MDMB-PINACA **(3)** and AMB-FUBINACA **(4)** to 5F-MDMB-PICA **(5)** and 4F-MDMB-BINACA **(7)** was observed following the legislative control of 5F-

MDMB-PINACA (3) and AMB-FUBINACA **(4)** in the People's Republic of China in August 2018, similar to changes noted recently in other jurisdictions worldwide. The evolution of the SCRA market in Scottish (and according to available data, wider UK) prisons, could be described as being relatively conservative, with little variability of compounds at any one time and emerging compounds for some time remaining almost exclusively within the indole/indazole-3-carboxamide structural class. Continued vigilance is required to track market trends of SCRAs whilst also taking all steps to reduce supply by ensuring the effectiveness of detection and screening systems is maintained and to minimise harm to drug users.

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Supplementary Information
Supplementary data to this article can be found online at:

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Table 1. A summary of the synthetic cannabinoid receptor agonist (SCRA) detected and their concentration ranges in 108 SCRA infused papers from 3 Scottish prisons

Compound	n	% of SCRA positive papers (number of samples)	Concentration Range (mg/cm^2)
5F-MDMB-PICA **(5)**	50	41 (59)	<0.08 – 0.76
5F-MDMB-PINACA **(3)**	39	29 (42)	<0.05 – 1.17
4F-MDMB-BINACA **(7)**	40	31 (45)	<0.09 – 0.94
AMB-FUBINACA **(4)**	3	3 (5)	0.20 – 1.16
MDMB-4en-PINACA **(8)**	19	15 (22)	<0.07 – 0.58
AMB-CHMICA **(6)**	1	1 (1)	0.58*

*Detected in a single card sample, later used for a whole sample concentration mapping study.

Table 2 Samples containing multiple synthetic cannabinoid receptor agonists (SCRAs)

			% of peak area of major SCRA detected							
Sample ID	**Date Seized**	**Major SCRA detected**	**5F-MDMB-PINACA**	**AMB-FUBINACA**	**5F-MDMB-PICA**	**4F-MDMB BINACA**	**AMB-CHMICA**	**MDMB-FUBINACA**	**CUMYL-4CN-BINACA***	**MDMB-4en-PINACA**
FL19/0067-2	23/11/18	**5F-MDMB-PINACA**	-	4.11	1.67	-	-	-	4.38	-
FL19/0078-2	11/02/19	**4F-MDMB-BINACA**	3.94	-	5.70	-	-	-	-	-
FL19/0110	28/04/19	**5F-MDMB-PINACA**	-	-	82.4-87.5	-	-	-	-	-
FL19/0111-5	01/05/19	**5F-MDMB-PICA**	66.9	-	-	-	-	-	-	-
FL19/0127	07/06/19	**4F-MDMB-BINACA**	-	-	-	-	54.7	35.5	-	-
FL19/0138-2	25/06/19	**4F-MDMB-BINACA**	-	-	16.2	-	-	-	-	-
FL19/0142	17/06/19	**MDMB-4en-PINACA**	-	-	-	4.5	-	-	-	-
FL19/0146	04/05/19	**4F-MDMB-BINACA**	-	-	17.6	-	-	-	-	-
FL19/0150	09/06/19	**MDMB-4en-PINACA**	-	-	-	51.4	-	-	-	-
FL19/0196	30/08/19	**4F-MDMB-BINACA**	-	-	-	-	-	-	-	48.5
FL19/0205	13/09/19	**4F-MDMB-BINACA**	-	-	-	-	-	-	-	48.7
FL19/0206-C	03/09/19	**4F-MDMB-BINACA**	-	-	-	-	-	-	-	68.0
FL19/0206-D	03/09/19	**4F-MDMB-BINACA**	-	-	-	-	-	-	-	81.4
FL19/0206-F	03/09/19	**4F-MDMB-BINACA**	-	-	-	-	-	-	-	55.1
FL19/0207-2	07/08/19	**5F-MDMB-PICA**	-	-	-	2.5	-	-	-	-
FL19/0210	18/09/19	**4F-MDMB-BINACA**	-	-	44.1	-	-	-	-	84.7
FL19/0215-E	18/09/19	**4F-MDMB-BINACA**	-	-	-	-	-	-	-	75.4
FL19/0215-F	18/09/19	**4F-MDMB-BINACA**	-	-	24.1	-	-	-	-	76.4
FL19/0215-G	18/09/19	**4F-MDMB-BINACA**	-	-	-	-	-	-	-	70.8
FL19/0224-1	04/09/19	**4F-MDMB-BINACA**	-	-	TRACE	-	-	-	-	38.4
FL19/0224-2	04/09/19	**4F-MDMB-BINACA**	-	-	TRACE	-	-	-	-	0.3
FL19/0232-2	23/09/19	**4F-MDMB-BINACA**	-	-	-	-	-	-	-	74.7

* tentative identification

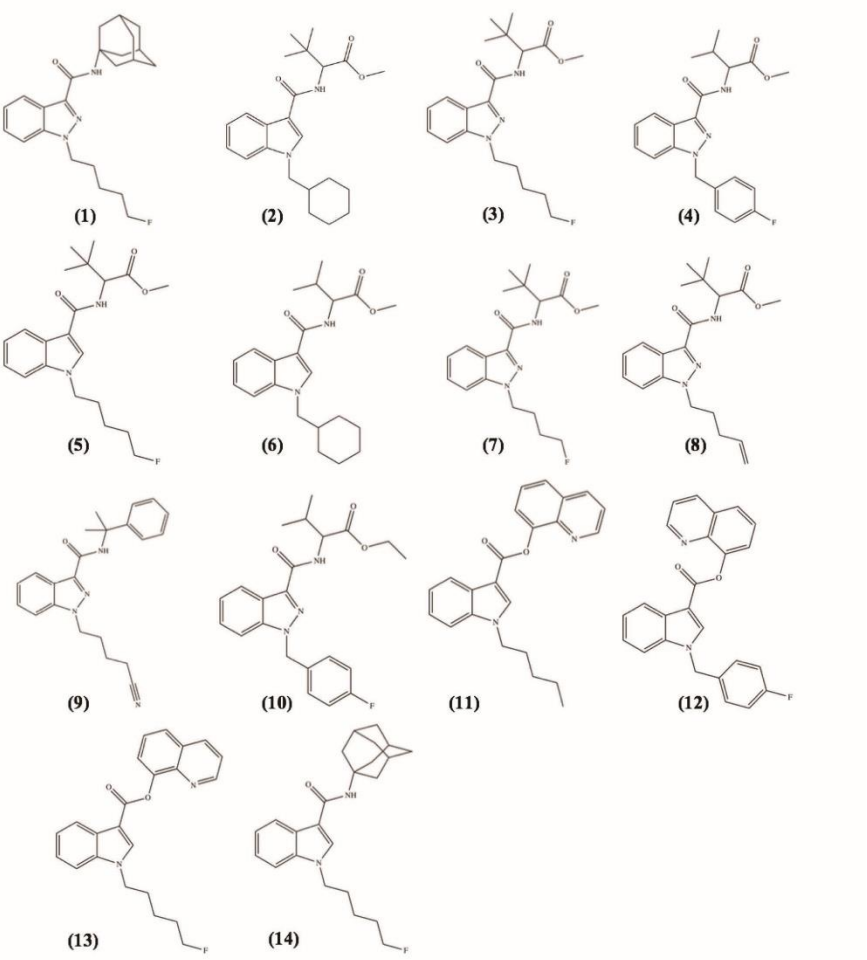


Figure 1. Relevant synthetic cannabinoid receptor agonist (SCRA) molecular structures: **(1)** 5F-APINACA (5F-AKB48); **(2)** MDMB-CHMICA; **(3)** 5F-MDMB-PINACA (5F-ADB); **(4)** AMB-FUBINACA; **(5)** 5F-MDMB-PICA; **(6)** AMB-CHMICA; **(7)** 4F-MDMB-BINACA; **(8)** MDMB-4en-PINACA; **(9)** CUMYL-4CN-BINACA; **(10)** EMB-FUBINACA; **(11)** PB-22 **(**QUPIC); **(12)** FUB-PB-22; **(13)** 5F-PB-22; and **(14)** 5F-APICA (STS-135).

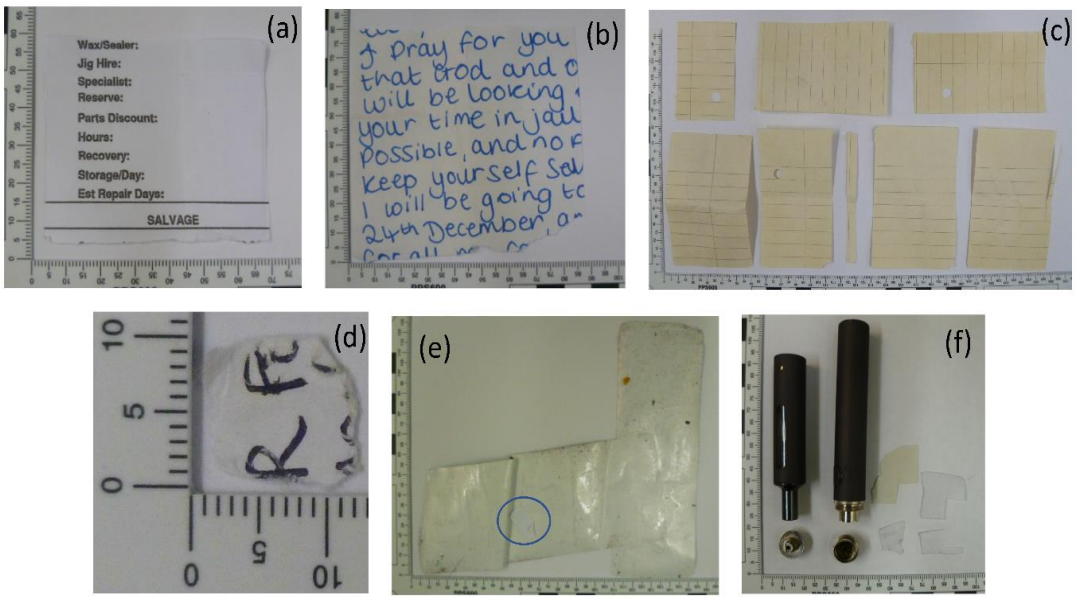


Figure 2. Examples of seized items submitted for synthetic cannabinoid receptor agonist (SCRA) analysis.
(a) paper sample FL19/0077; (b) paper sample FL19/0064; (c) multi-part paper sample FL19/0149; (d) a typical single dose (approx. 1cm^2) paper sample, FL19/0111-7; (e) sample FL19/0082: paper stuck to underside of stuck together milk bottle labels, most likely to facilitate exchange of a SCRA paper dosage unit; (f) sample FL19/0091: Disassembled e-cigarette seized with papers

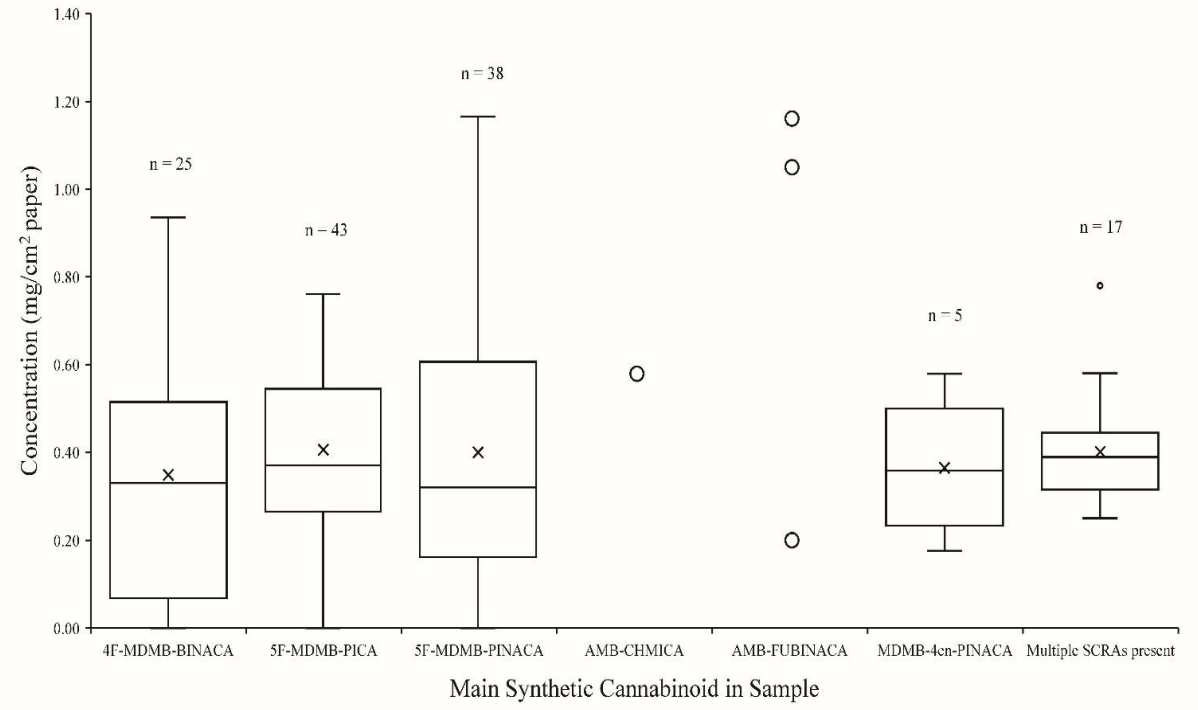


Figure 3. Concentrations of the main synthetic cannabinoid in the infused paper samples from three Scottish prisons found positive for one or more synthetic cannabinoid (n=132*).

*Fourteen samples were not quantified as they were only present at trace levels in the qualitative analysis or not enough sample was remaining for quantitative analysis.

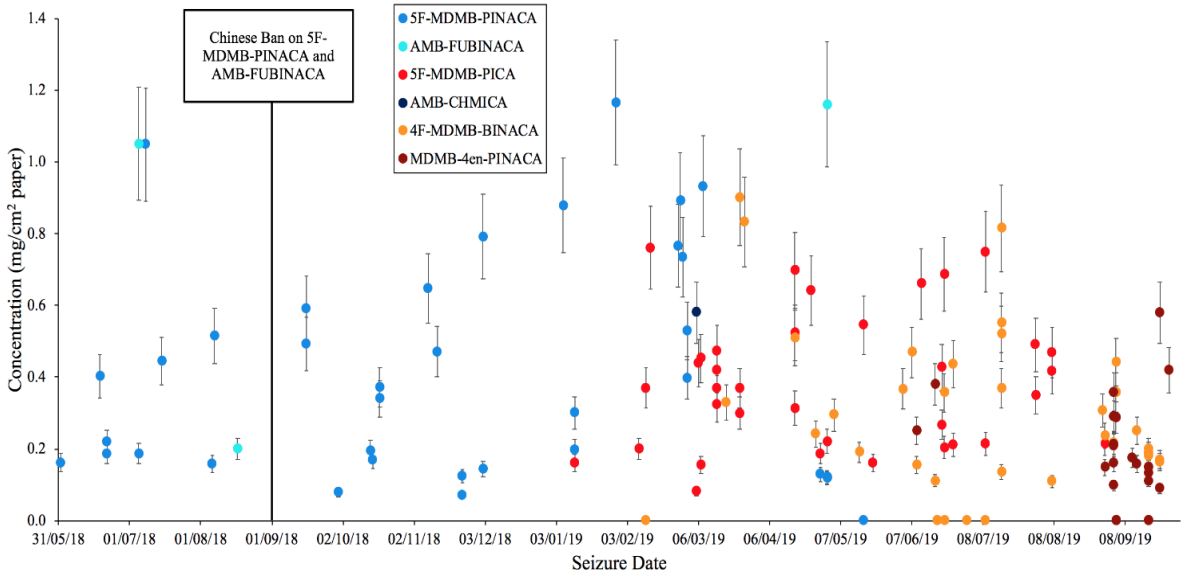


Figure 4. Timeline of the main synthetic cannabinoid concentrations of all quantitated samples with a seizure date from three Scottish prisons (n=137) where error bars represent the estimated error of 15% from the method validation performed. Any samples on the x-axis (indicating a concentration of 0) had concentrations below the limit of quantitation (<0.05-0.09 mg/cm^2).

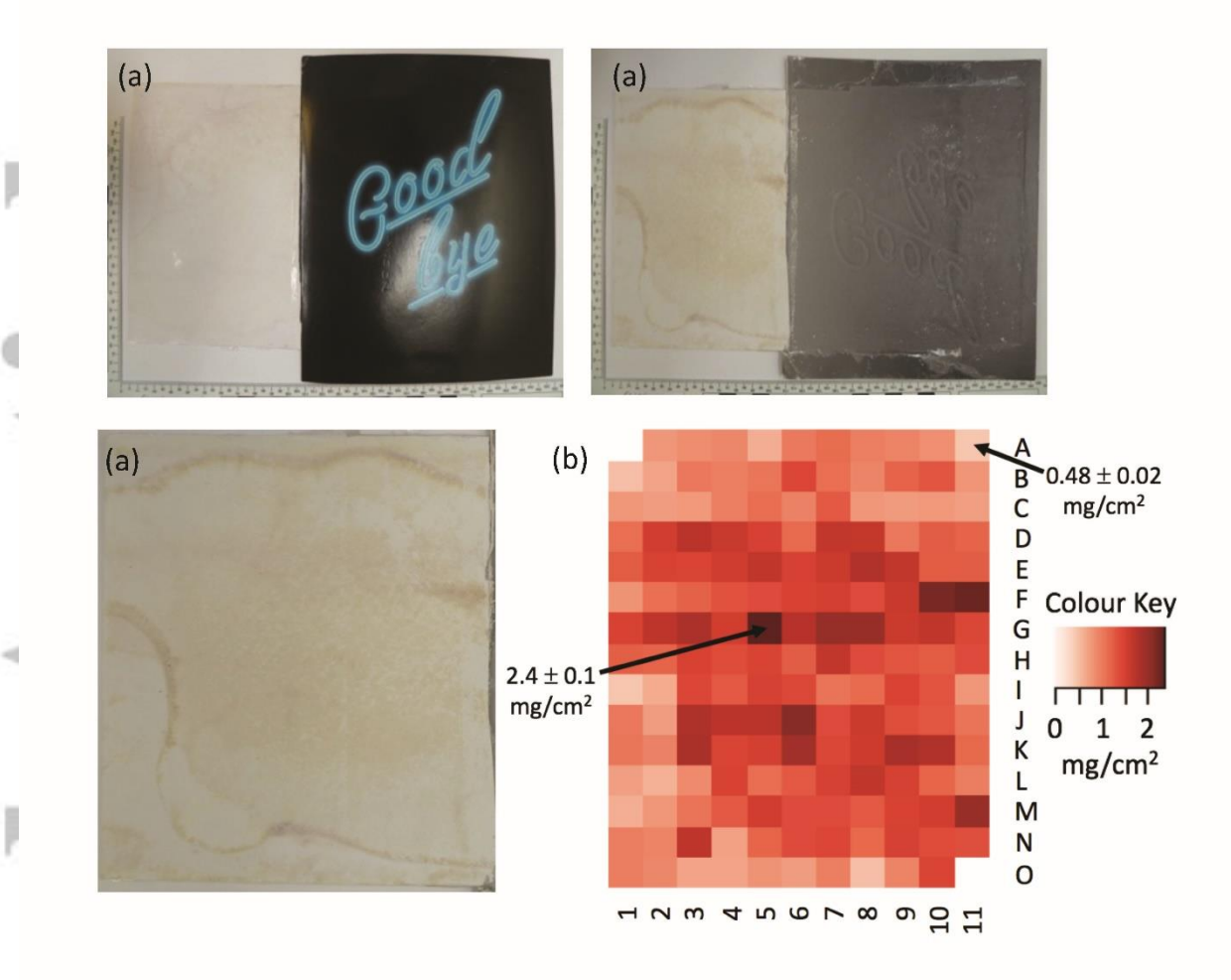
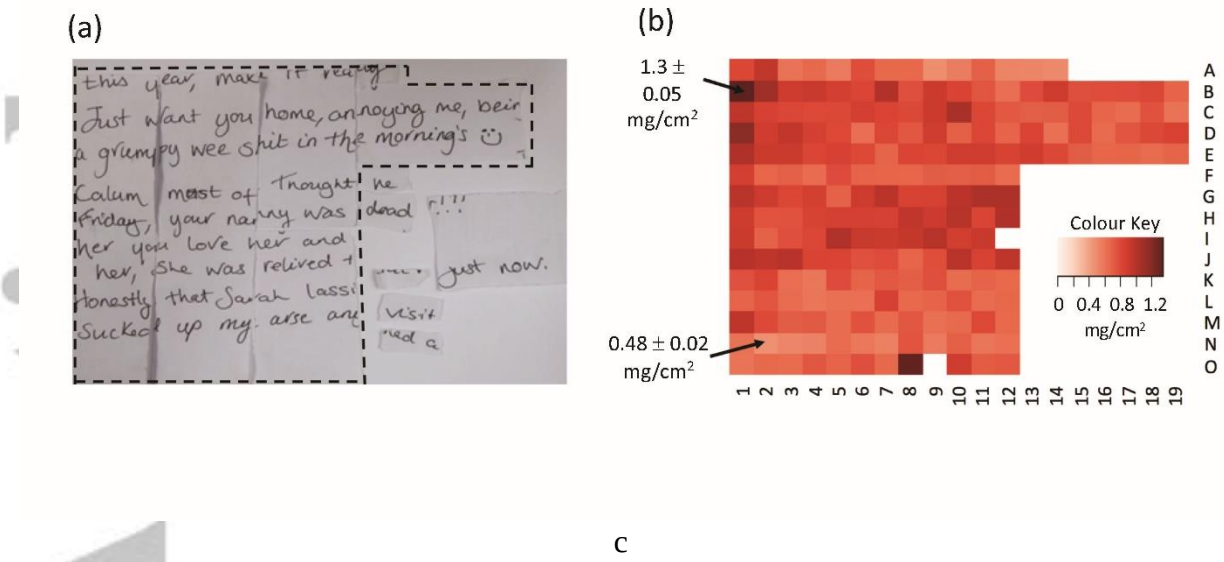


Figure 5. (a) Sample FL19/0097: greetings card with white card in interior; **(b)** AMB-CHMICA concentration mapping across paper (white squares in opposite corners indicate positions of samples taken for initial qualitative (screening) analysis).



c

Figure 6. (a) Sample FL19/0100 from Prison 1: cut up note; **(b)** cut up note showing positions of six pieces which formed a physical fit and were used in the 5F-MDMB-PICA concentration mapping; **(c)** 5F-MDMB-PICA concentration mapping across paper (white squares indicate positions of samples taken for initial qualitative (screening) analysis).

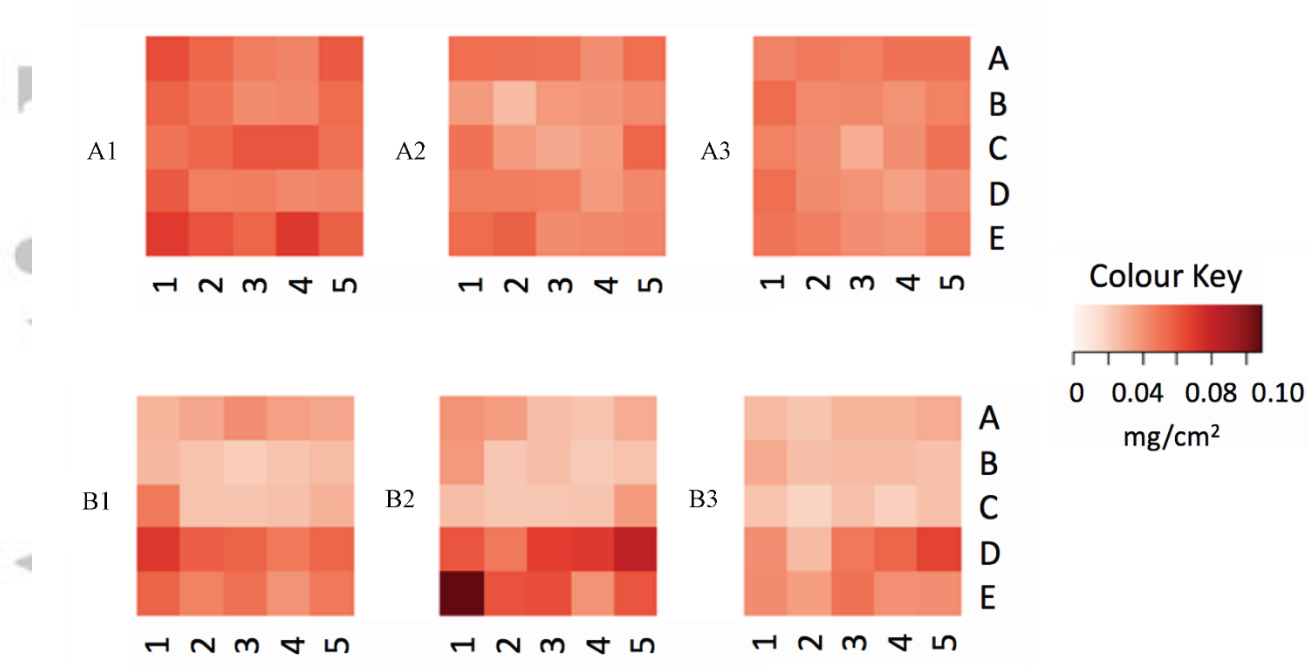
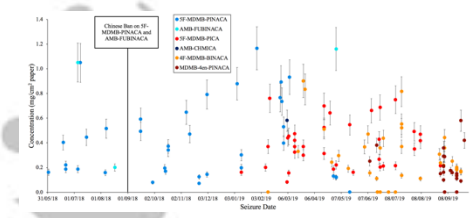


Figure 7. Laboratory prepared SCRA infused paper samples. Six 25cm^2 pieces of lined notepaper were placed flat in 5 mL of an approximately 4 mg/mL solution of (*R*)-5F-MDMB-PINACA for approximately 10 seconds. Replicate samples A1-3 were removed from the solution and dried flat for one hour. Replicate samples B1-3 were removed from the solution and hung up to dry for one hour with the top of the sheet marked in pencil.

Synthetic Cannabinoid infused paper samples seized in Scottish prisons were qualitatively and quantitatively analysed and the market evolution described from June 2018 to September 2019.



Detection and quantitation of synthetic cannabinoid receptor agonists in infused papers from prisons in a constantly evolving illicit market

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